ABSTRACT

The present invention describes a rapid and efficient *in vivo* library-*versus*-library screening strategy for identifying optimally interacting pairs of heterodimerizing polypeptides. It allows for the screening of a protein library against a second protein library, rather than against a single bait protein, and thus has numerous applications in the study of protein-protein interactions. Additionally, it allows for the application of different selection stringencies. Two leucine zipper libraries, semi-randomized at the positions adjacent to the hydrophobic core, were genetically fused to either one of two designed fragments of the enzyme murine dihydrofolate reductase (mDHFR), and cotransformed into *E. coli*. Interaction between the library polypeptides was required for reconstitution of the enzymatic activity of mDHFR, allowing bacterial growth. Analysis of the resulting colonies revealed important biases in the zipper sequences relative to the original libraries, which are consistent with selection for stable, heterodimerizing pairs. Using more weakly associating mDHFR fragments, we increased the stringency of selection. We enriched the best performing leucine zipper pairs by multiple passaging of the pooled, selected colonies in liquid culture, as the best pairs allowed for better bacterial propagation. This competitive growth allowed small differences among the pairs to be amplified, and different sequence positions were enriched at different rates. We applied these selection processes to a library-*versus*-library sample of 2.0×10^6 combinations, and selected a novel leucine zipper pair which may be appropriate for use in further *in vivo* heterodimerization strategies.